Experiment 1

Date :

USE AND CARE OF THE COMPOUND MICROSCOPE

AIM

To study the parts, uses and care of the compound light microscope.

PARTS OF THE COMPOUND MICROSCOPE ARE:

A. Mechanical parts
B. Optical parts

A. Mechanical Parts

Include a foot or base, an arm, a body tube, a stage and a sub stage.

The base is triangular or rectangular shaped, to give the microscope maximum stability. It supports the microscope on the worktable.

The arm is attached to the base. The arm carries the body tube in its upper parts and the stage and sub stage in its lower part.

The body tube is attached to the arm. The body tube carries the eye piece in its upper part and the revolving nosepiece in its lower part. The nose piece carries the three objectives. The coarse and fine adjustment screws are used to bring the object in to focus.

The stage is a rigid platform provided with a slide holder to keep the slide in position. There are two screws for moving the slide forwards and sideways. The stage can be moved up or down by means of coarse and fine adjustment screws. The stage has an aperture in its centre. Light passes through the aperture and through the slide into the objective lens of the microscope.

The sub stage below the stage carries the condenser and iris diaphragm.

B. Optical parts

Include the magnifying parts and illuminating parts.

Magnifying parts are the eyepiece and objective lenses. The eyepiece fits into the top of the body tube. Usually two magnifications are available for the eyepiece -5x and 10x. 5x is used when lower magnification is required and 10x when higher magnification is needed. The purpose of the eyepiece lens is to magnify the image formed by the objective lens.

The objective lenses are attached to the lower end of the revolving nose piece.

There are three objective lenses.

1. The low power objective lens is of magnification 10 (10x). Working distance is 16 mm. the field under view is large.
2. The high-power objective has a magnification of 40 (40x). The working distance is 4 mm. this is used for more detailed examination of a particular area.
3. The oil immersion objective has a magnification of 100(100x). The working distance is 1.8 mm. Here a thin layer of air exists between the slide and objective lens. As light passes through this thin layer of air (rarer medium) form glass (a denser medium) refraction of light rays will occur and very little light enters the objective, resulting in blurred image. To prevent this, oil having the same refractive index as that of glass is used to replace the air between the slide and objective lens. Oil such as cedar wood oil, paraffin oil, Canada balsam, DPX, etc is used for this purpose. In our lab we use cedar wood oil.

The revolving nosepiece allows any objective to be swung into position, being indicated by a click. Usually in newer microscope if one objective is on focus, the other objectives will be more or less in focus, when switched into position and requires only fine adjustment. This arrangement of objectives is called par focal arrangement.

The illuminating parts include the condenser, iris diaphragm and the electric lamp

The condenser lens is used converge the light rays reflected from the mirror, towards, the object. It can be moved up and down by means of a screw. Usually it has two lenses mounted in a short cylinder. The parallel beam of light is converged into a cone of light, which passes through the aperture of the fixed stage.

The iris diaphragm is placed below the condenser and is used to control the amount of light entering the microscope. It can be closed or opened by means of lever.

The electric lamp is present at the base of the microscope as a light source. It has an on -off switch and a knobe adjust the brightness.

Image Formation

The microscope can be considered to be composed of two simple convex lenses, the objective lens and eyepiece lens.

The objective lens forms a real and inverted magnified image of the object. The eyepiece lens further magnifies the image so that a virtual magnified image of the object is formed.

Magnification by the microscope

Magnification produced by the microscope is equal to objective magnification multiplied by the eyepiece magnification.

With a low power objective of magnification 10 and an eyepiece of magnification 10, the total magnification is 10 x 10 = 100 times. High power objective with magnification of 40 gives a total magnification of 40 x 10 = 400 times. Oil immersion objective of magnification 100 produces a total magnification of 1000 times.

Adjustment of microscope in the microscope used in our lab

Low Power : The low power objective (10 x) is brought in line with the eyepiece lens. The stage is lowered, condenser is lowered, iris diaphragm is partially opened, and brightness is adjusted to lowest setting. Looking through the side, stage is raised as much as possible. Then looking through the eyepiece, lower the stage slowly till the image becomes clear.

High Power : High power objective is brought into position. Condenser is half raised the iris diaphragm is 3/4th opened. Looking through the side, raise the stage. Looking through the eyepiece, lower the stage slowly till the image becomes clear.

Oil immersion : The oil immersion objective is brought in position. Condenser is raised; iris diaphragm is fully opened; brightness is adjusted to highest setting. Put a drop of oil on the slide. Looking through the side raise the stage till the oil immersion objective touches the oil. Now looking through the eyepiece lower the stage slowly till a clear image is obtained. Never raise the stage while looking through eyepiece, it will crush objective.

Care of the microscope

1. The microscope should be kept at room temperature, protected from dust and not exposed to direct sunlight.
2. It should be cleaned regularly and mechanical parts kept free of dust.

3. Optical parts should be cleaned only with great care so that they do not get scratched. Dust or grease should be removed by lightly wiping the lens with specially prepared soft lens paper or well washed silk or soft cloth. The oil adhering to the oil immersion objective should be immediately removed by means of lens paper.
4. Never hold the microscope by its body tube. It is safe to carry it with two hands, one holding the side arm and the other supporting under the base.

Microscope was invented by Anton Von Leuwenhoek. Other types of microscopes are dissecting microscope, dark ground microscope, polarizing/ interference microscope, fluorescence microscope, phase contrast microscope, transmission electron microscope (TEM), scanning electron microscope (SEM) etc. Study resolving power, numerical aperture, and aperture. TEM is used to analyze ultra structural details at high resolution. SEM is used to analyze details of topography at high resolution. We cannot directly see using electron microscope because eyes do not detect electrons. Electron microscope can produce photographs. Preparation of specimen for electron microscopy itself causes its death.

Questions

1. In a tabular form, compare the adjustments for low power, high power and oil immersion view.
2. Why is cedar wood oil used for oil immersion view?

1)	MAGNIFICATION	POSITION OF CONDENSOR	IRIS DIAPHRAGM	WORKING DISTANCE
LOW POWER	10 x10 =100	Lowest	half open	16mm
HIGH POWER	40 x10 =400	half raised	$\frac{3}{4}$ open	4mm
OIL IMMERSION	100 x10 =1000	fully raised	fully open	1.8mm

2) Cedar wood oil is used for oil immersion because it has the same refractive index as that of glass that is used to replace the air between the slide and objective lens.

Experiment 2 Date :

MICROSCOPIC EXAMINATION OF BLOOD

AIM

To study the RBCs under the microscope and note their morphological changes when placed in isotonic, hypotonic and hypertonic saline solutions.

APPARATUS

Glass slides, cotton, spirit, sterile lancet, normal saline, hypertonic saline, hypotonic saline. Any concentration above 0.9% sodium chloride is hypertonic.

PROCEDURE

Technique of finger Pricking

Finger pricking is done using a needle with flat body (lancet) or a spring loaded automatic lancet.

Before pricking ensure that all equipments are ready

Site : The palmar surface of distal phalanx of middle or ring finger is preferred. Pricking the thumb or little finger can lead to radial or ulnar bursitis.

Preparing site : Clean the site with spirit. Shake the hand to increase circulation. Milk down the finger and then clamp off at the distal crease to block off venous blood. Allow the spirit to dry so that a compact drop can be formed.

Pricking : Holding the finger steady do a single quick stab on the ball of the finger. Finish it off with a single strong confident prick, do not do multiple pricks that are ineffective.

Collection of blood drop: Wipe away the first drop and wait for a large round drop to collect. Gentle squeezing in permissible. The squeezing has to be gentle or else there will be mixing with tissue fluid, resulting in false values. Also pressure can close the wound. When a drop is formed collect it immediately or it will clot.

Examination of fresh blood

Take a clean glass slide. Sterilize the fingertip using a piece of cotton dipped in rectified spirit. Prick the fingertip using a sterile lancet. Place a small drop of blood on the glass slide. Put the cover slip and examine it under the microscope. First examine under low power, and then shift to the high power.

Blood in isotonic saline

Take a drop of isotonic saline on a clean glass slide. Add a small drop of blood to it. Mix and put a cover slip. Examine under high power objective.

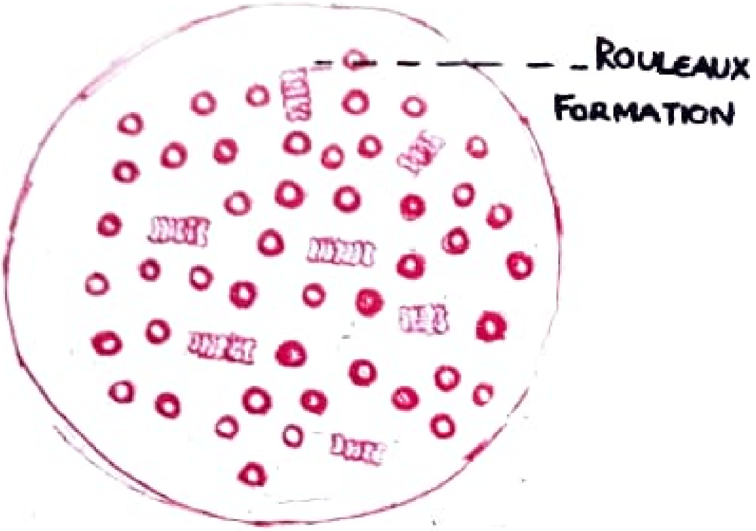
Blood in hypotonic saline

On a clean glass slide, take a drop of hypotonic saline. Add a small drop of blood to it. Mix and examine under high power of the microscope.

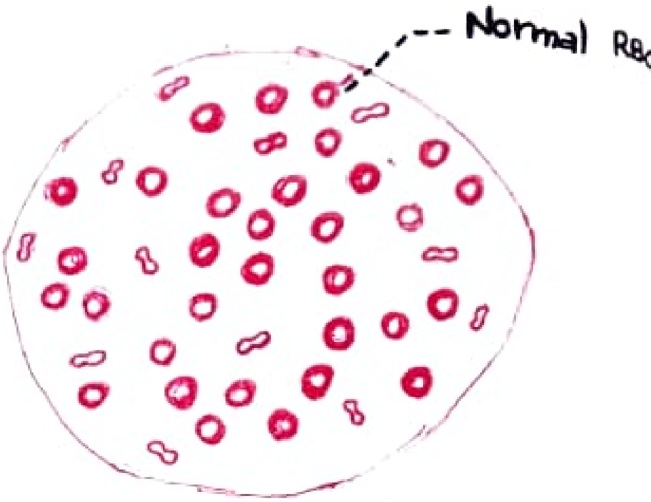
Blood in hypertonic saline

On a clean glass slide mix a drop of blood with one drop of hypertonic saline. Examine under high power.

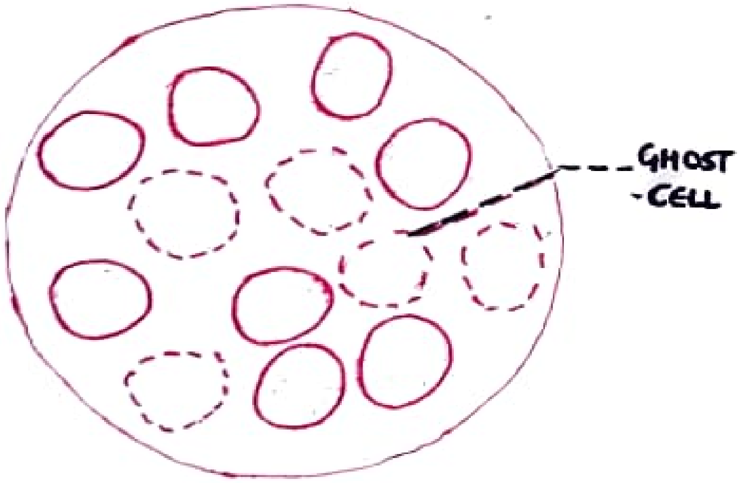
FRESH BLOOD



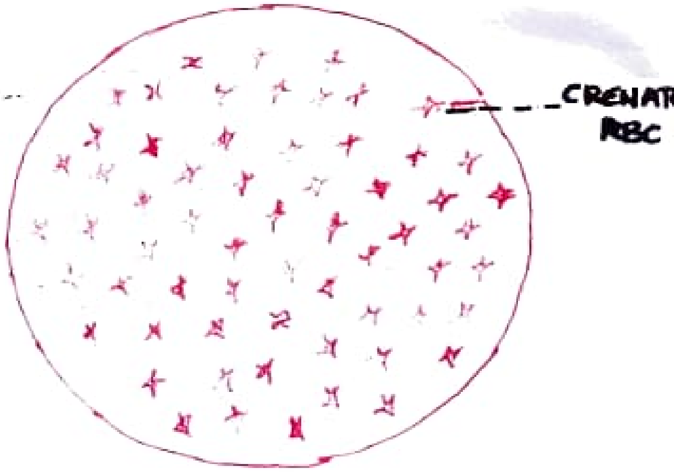
ISOTONIC SALINE



HYPOTONIC SALINE



HYPERTONIC SALINE



OBSERVATIONS:

1. When the blood drop is examined as such, RBCs are seen arranged in piles with their flat surfaces facing each other, this is known as rouleaux formation.
2. Blood in isotonic saline: Here RBCs are seen separated without much change in their morphology. This is because isotonic saline has got the same osmolality as that of the intracellular fluid of the RBC.
3. Blood in hypotonic saline: Here some of the RBCs are swollen and some others are seen ruptured. This is because fluid from outside enters the cell, causing it to swell and then rupture. This rupture of RBC is known as haemolysis and the cell ruptured in the process becomes the ghost cell.
4. Blood in hypertonic saline: Here fluid from RBC passes out because of the high osmolality outside. As a result, the cell membrane shrinks and the cell wall has wrinkled appearance. The cells are called crenated cells.

Questions

1. Define endosmosis and exosmosis.
2. What are the factors affecting rouleaux formation?
3. Draw the observations

1. (a) Endosmosis

Endosmosis is the movement of water into the cell when the cell is placed in a hypotonic solution. The movement of water causes the cell to swell. An RBC kept in a hypotonic solution swells and undergo hemolysis

(b) Exosmosis

Exosmosis is the movement of water out of the cell when the cell is kept in a hypertonic solution. The cell becomes verflaccid by the movement of water. An RBC kept in a hypertonic solution shrinks

2. Factors affecting rouleaux formation are

(a) Plasma proteins:- Fibrinogen and globulins neutralise the surface charges of RBC and this increases rouleaux formation, but increase in albumins decrease rouleaux formation.

(b) Viscosity of blood:- Increase in viscosity decrease the rate of rouleaux formation

(c) Body temperature:- Increase in body temperature increases the rate of rouleaux formation.

Experiment 3 Date :

PACKED CELL VOLUME OR HAEMATOCRIT

AIM

To determine the packed cell volume of the given sample of blood.

PRINCIPLE

When a known volume of anticoagulated blood is centrifuged, as the cells are heavier, they settle down, leaving a clear column of plasma above.

APPARATUS

Wintrobe's haematocrit tube; Pasteur pipette or syringe with L.P. needle, oxalated venous blood, sterile needle, spirit, cotton.

Wintrobe's haematocrit tube is a special, thick walled glass tube, 11cm long, with an internal diameter of 2.5 mm and a flat inner base. It is graduated from 0 to 10 in both directions. The graduation from 0 to 10 above downwards is used to estimate ESR by Wintrobe's method.

Venous blood mixed with double oxalate mixture which is the anticoagulant used for PCV. Double oxalate mixture is a mixture of ammonium oxalate and potassium oxalate in the ratio 3:2. Double oxalate is used, as it will not alter the morphology of the cell. Ammonium oxalate produces swelling of the RBC and potassium oxalate produce shrinkage of RBC. Their actions are counter balanced, when present in the above ratio.

PROCEDURE

After thoroughly mixing the anticoagulated blood sample, about 1 – 1.5 ml is taken up into a Pasteur pipette. The tip of the pipette is lowered into the haematocrit tube until it touches the bottom. The pipette is slowly withdrawn while the blood is transferred into the haematocrit tube. The tube is filled without any air bubbles, exactly up to the 0 mark, at the top of the tube. Then close the tube using a cotton plug to prevent evaporation. Then it is centrifuged at a speed of 3000 revolutions per minute, for 30 minutes. The height of the column of packed red cells upto the bottom of buffy coat is directly read from the haematocrit tube and is multiplied by 10 to express as percentage.

Automated cell counters calculate PCV using MCV.

OBSERVATION

The red cells are seen packed at the bottom and the straw-colored column of plasma is seen above that. In between there is a greyish white layer consisting of WBCs and platelets. This layer, called the buffy coat, is 0.5 – 1 mm in thickness.

DISCUSSION

P.C.V. is the ratio of the volume of red cells to the volume of whole blood, expressed as percentage.

Normal values: Male = 45 ± 7%

Female = 42 ± 7%

VARIATIONS

(a) High P.C.V:

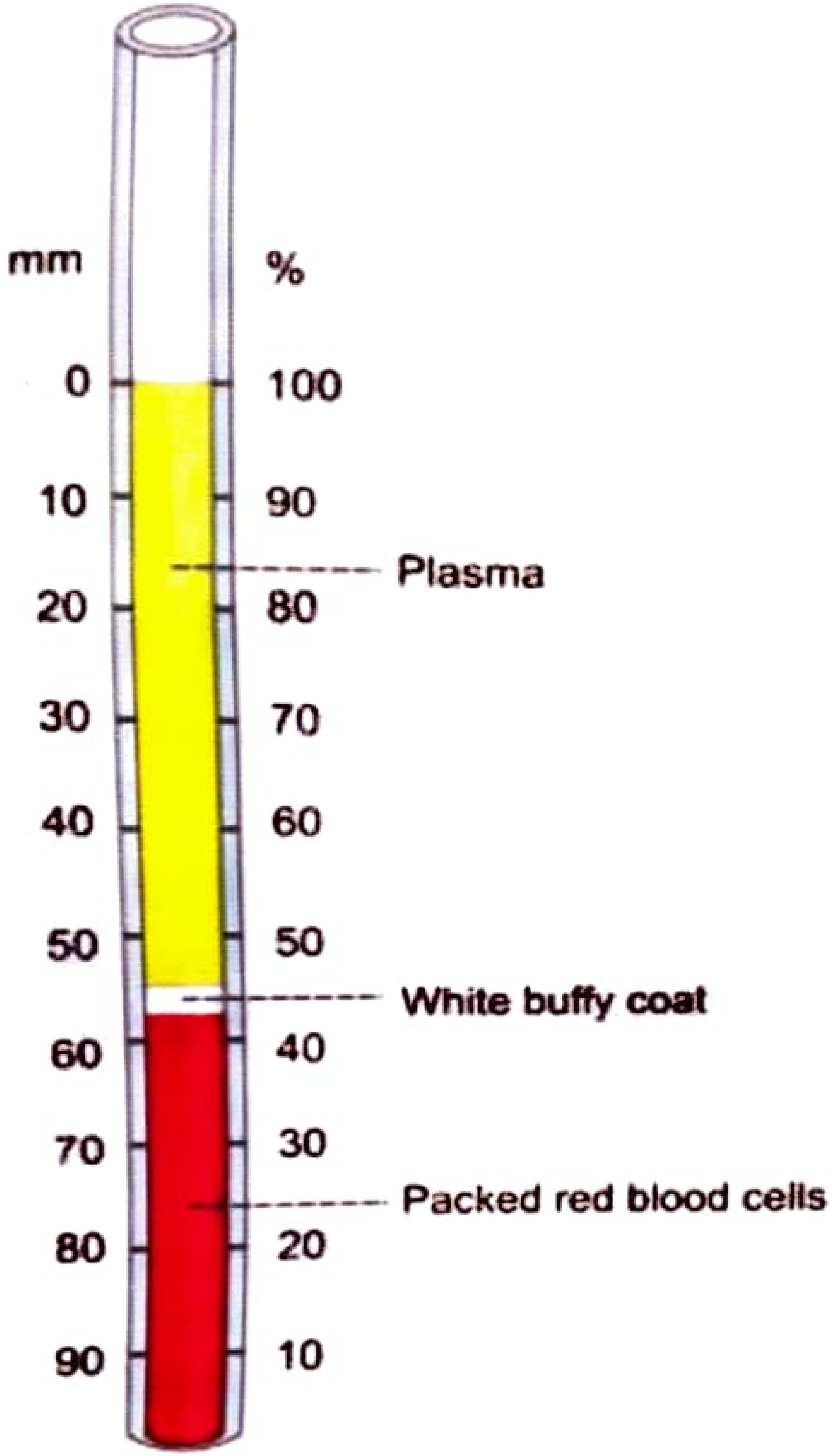
Physiological conditions :

(1) New born

(2) After exercise

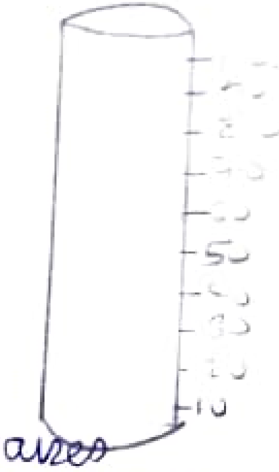
(3) High altitude

(4) Dehydration resulting from profuse sweating without adequate fluid intake.


mm
%
Plasma
White buffy coat
Packed red blood cells

complete

1) Wintrobe's haematocrit tube is also known as Westergren tube. It measures the rate at which RBCs settles out of plasma. It is used in differentiation of packed cell volume (PCV) and determination of Erythrocyte sedimentation (ESR).



2. As blood enters the capillary, fluid passes into the tissues and lymphatic capillaries. As this blood flow enter the venules, most of the extracellular fluid is reabsorbed. Hence the higher values for haemoglobin, haematocrit in venous blood.

3. It is a procedure for determining the ratio of the volume of packed RBC to the volume of whole blood by centrifuging a minute quantity a blood in a capillary tube coated with heparin.

4. A higher than normal haematocrit can indicate
(a) Dehydration
(b) Polycythemia vera, a type of blood ~~fluids~~ through cancer where bone marrow makes too many red blood cells.
(c) lung or heart disease.

5. Relative polycythemia?

Pathological conditions :

(1) Polycythemia Ruba Vera

(2) Dehydration resulting from severe gastroenteritis

(3) Burns leading to haemoconcentration

(b) Low P.C.V.:

Physiological condition:

(1) Pregnancy – PCV is low due to haemodilution

(2) In females

Pathological conditions :

In various types of anemias

Plasma column :

It is normally straw colored. Reddish tinge indicates haemolysis. It is yellow in jaundice, milky and opaque in lipemia.

Buffy coat :

Thickness of buffy coat gives a rough estimate of leucocyte count. 1mm thickness corresponds to 10000 WBCs. In leukemia, the thickness is increased. Buffy coat can be used for LE cell test for SLE. Smears from buffy coat are used to detect plasma cells and blast cells (in those leukemia's where circulating blast cells are few). QBC test (Qualitative buffy coat analysis) can be used to detect malarial parasite more easily than by peripheral blood smear examination.

Source of error:

1. Inadequate mixing of the blood sample
2. Irregularity in the bore of the tube
3. Error in centrifugation

Uses of PCV :

1. To detect anemia and hemoconcentration
2. To detect abnormalities by observing plasma column.
3. To detect abnormalities using buffy coat
4. To calculate MCV and MCHC.

Result:

PCV of the given sample of blood is-..34.....%.

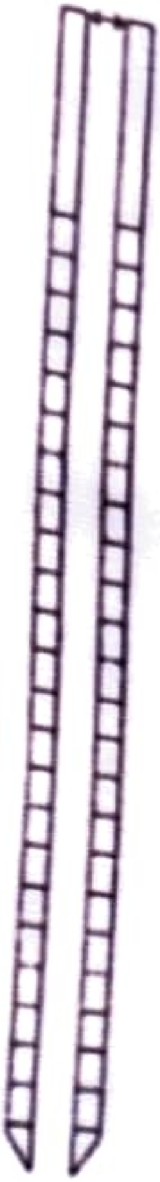
Questions-

1. Draw and label Wintrobe's hematocrit tube. What are it's uses?
2. Why is PCV increased in venous blood?
3. What is Microhematocrit?
4. What is the effect of a high haematocrit?
5. What is absolute and relative polycythemia? List some causes for each.

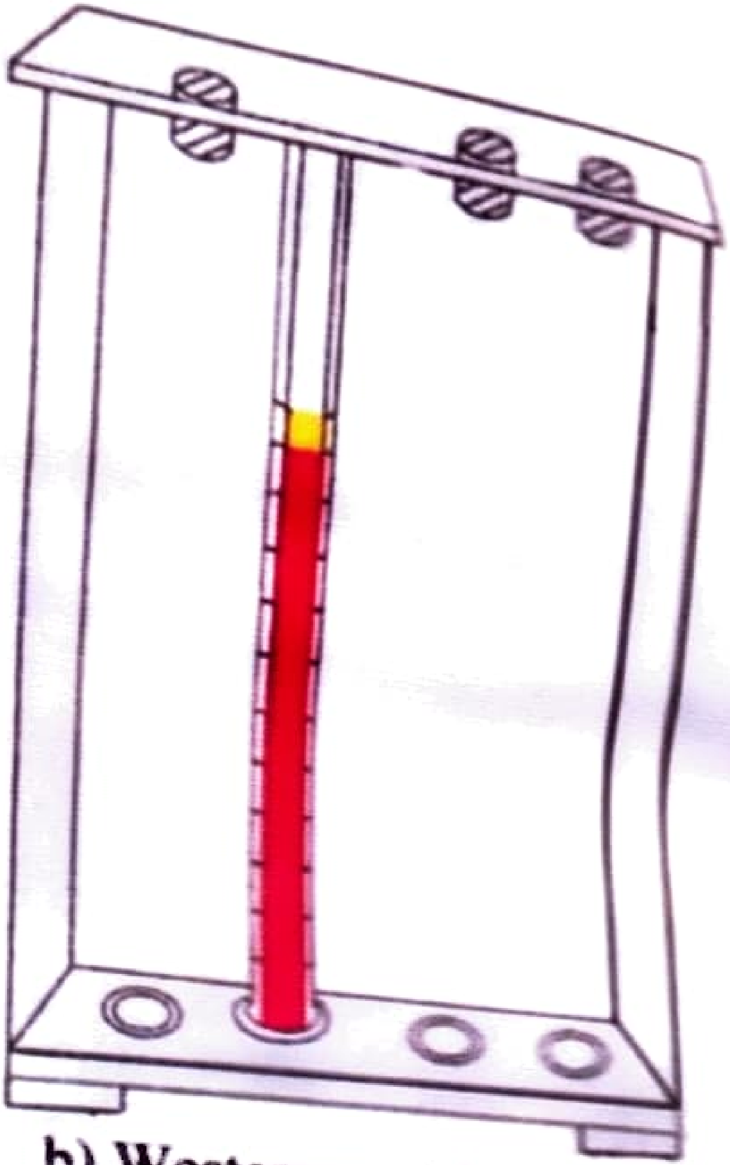
- RBC count remains the same but plasma level decreases
- It can be caused by loss of body fluids, through dehydration, sweating, etc.

Absolute polycythemia.

- RBC count increases.
- It can be caused by various pathological reasons such as hypoxia, high altitude, sickle cell anemia, etc



Westergren tube



b) Westergren tube on the rack

Experiment 4 **Date :**

ERYTHROCYTE SEDIMENTATION RATE

AIM

To determine the Erythrocyte Sedimentation Rate (ESR) of the sample of Blood. ESR of a sample of blood.

PRINCIPLE

ESR is measured as the length in millimeters of the clear column of plasma that collects at the top of a vertical column of anticoagulated blood kept undisturbed for one hour.

APPARATUS

Westergren's pipette, Westergren's stand, cotton.

Westergren's pipette is a long cylindrical thick glass tube of 300mm length and 2.5mm bore diameter. It is open at both ends and is graduate from 0 to 200 in mm scale from top to bottom at 1mm interval.

Westergren's stand can hold vertically (six) more than one pipette at a time.

PROCEDURE

Westergren's method

Take anticoagulated blood in a small beaker. 3.8% Trisodium citrate is the preferred anticoagulant and is used in the ratio 1:4 with blood. A clean dry pipette is taken and its lower end is introduced well below the surface of the blood. Draw blood in to the pipette without any air bubbles, with the help of a syringe attached via a rubber tube to the top of the pipette. Using cotton, wipe off the blood sticking to the outer surface of pipette. Keep the upper level of blood column exactly at zero mark and fix the pipette vertically on the stand. Wipe off any blood remaining in the rubber stopper on which the pipette is kept. Keep the pipette undisturbed for one hour. Take the reading directly from the pipette at the end of the first hour.

DISCUSSION :

Normal value by Westergren's method

Males = 3 -5 mm / 1st hr

Females = 4 -7 mm / 1st hr

ESR is the rate at which erythrocytes sediment in the first hour in vertical column of anticoagulated blood when kept undisturbed. RBCs settle down due to their greater specific gravity than that of plasma.

Wintrobe's method: Inferior to Westergren's method. Wintrobe's tube and Wintrobe's rack are used. The 0 -10 markings above downwards are used.

ESR using Esrite Kit

Esrite Kit - consists of Esrite glass single use filling vials, stand and anodised scale with marking in mm. The grounded tips of the borosilicate Esrite glass tube fits into the plastic Esrite vials containing blood to form a leak-proof system. These can be kept snugly in the cavities in the front part of the stand. The anticoagulant used in sodium citrate 3.8% and proportion of anticoagulant to blood in 1:4.

PROCEDURE

Place the stand on a level surface and insert the scale into the slot provided to the full depth. Fill the vials with anticoagulated blood and introduce the vials into the cavities provided on the stand.

Vertically introduce grounded tip of the Esrite tube into the vial and gently push down till upper meniscus corresponde to zero. Read out the length of the clear column at the end of 60 minutes.

Factors Influencing ESR

1. Rouleaux formation is a very important factor on which ESR depends. When degree of rouleaux formation is more (the ratio of surface area to mass of RBC is less) ESR is more. Rouleaux formation is influenced by different factors like:
   - (i) Cellular factors
     - (a) Shape of RBC – Biconcave disc shape favours rouleaux formation
     - (b) Size of RBC: When more, sedimentation is faster
     - (c) RBC count: Increase in RBC count decreases ESR
   - (ii) Plasma Factors
     - (a) Albumin reduces rouleaux formation
     - (b) Globulin, and fibrinogen favour rouleaux formation
     - (c) Products of tissue destruction and inflammation (eg. C-Reactive Protein) favour rouleaux formation. Plasma proteins affect rouleaux formation by changing the electrical charges on the red cells.
     - (d) Cholesterol increases ESR. Phospholipids decrease ESR.
2. Technical factors: Increased temperature, and inclination of pipette increases ESR. Sedimentation rate decreases after first hour. Type of anticoagulant used also affects ESR.

Conditions in which ESR value is increased.

1. Physiological conditions like menstruation, pregnancy.
2. Pathological conditions:
   - (a) Rheumatic fever, rheumatoid arthritis, tuberculosis, malignancy (eg. Multiple myeloma), acute inflammatory diseases, acute myocardial infarction, conditions of severe tissue breakdown such as burns, trauma, surgery, etc.
   - (b) Anemias, except some types.
   - (c) Nephrosis

Decrease in ESR value

1. Physiological in new born
2. Polycythaemia
3. Spherocytosis, sickle cell anemia, thalassemia
4. Afibrinogenemia

Clinical significance of ESR

In the past clinical importance was given to ESR as a diagnostic and prognostic tool in the assessment of several pathological conditions like rheumatic fever tuberculosis etc. Abnormal ESR value is not diagnostic of any disease condition but with other evidence it helps in a diagnosis. ESR has a more prognostic significance i.e., it helps in assessing the course taken by a disease in response to treatment. E.g. tuberculosis, collagen diseases.

PRECAUTIONS

1. There should not be any air bubble in the blood column
2. The top level of blood column should exactly correspond to zero level
3. The pipette should be mounted vertically
4. Reading should be taken exactly at the end of first hour because rate of sedimentation decreases after first hour.
5. The pipette should be exactly vertical. Rate of sedimentation increases when the pipette is slanting.

Other methods

1. Wintrobe's method
2. Barrett's micro ESR method
3. Zeta sedimentation rate (ZSR) using zetafuge. It requires only very small quantity of blood.

Result :

Questions-

1. What is Zeta potential?
2. What are the factors affecting ESR?
3. Why do the red cells settle down in a sample of anticoopulated blood?
4. Why is ESR reading taken after one hour?
5. What are the stage of ESR?

1) Zeta potential is defined as the degree of negative charge on the surface of a red blood cell, it is the potential difference between the negative charges on RBCs and the cations in the fluid portion of blood.

2) Factors affecting ESR are
(a) Physiological factors
- Plasma factors
- Red cell.
- Rouleaux formation.
- Pregnancy.

(b) Laboratory factors
- Temperature
- Anticoagulants.
- Time
- Tube factor

3. When the sample is allowed to stand in the vertical tube for 1 hour. The red blood cell will slowly fall (settle) to the bottom due to the effect/influence of gravity. This will leave a clear, straw-coloured fluid at the top of tube.

4. ESR reading is taken after one hour became ESR stability degrades overtime due to changes in the shape of the red blood cells which become sphere. These spherical cells are difficult to aggregate affecting rouleaux formation. In addition, the interaction of charges between the membrane surface of RBC & the plasma protein is modified

5) Stages of ESR are
(a) Stage of Rouleaux formation
(b) Stage of sedimentation or settling
(c) Stage of packing

Experiment 5 **Date :**

HAEMOGLOBINOMETRY

AIM

To estimate the hemoglobin content in a sample of blood by Sahli's method.

PRINCIPLE

Hemoglobin present in the sample of blood is converted into acid haematin and the colour developed is compared with the given standard.

APPARATUS

Sahli's haemoglobinmeter (haemometer), distilled water, rectified spirit, cotton, lancet.

Sahli's haemoglobinometer consists of -

1) Comparator box : with two colour standards, one on either side with a central slot for keeping the diluting tube. The standard is non fading yellowish brown tinted glass, the colour of which is that of acid haematin obtained by treating blood containing 14.5 gm of haemoglobin per deciliter, with 0.1 N HCl, and diluting it 100 times.
2) A special diluting tube which is graduated in gram/dl scale on one side and percentage scale on the other side, (14.5 gm/dl corresponding to 100%).
3) Haemoglobin pipette, which is a capillary pipette with a 20 cu.mm marking.
4) A glass stirrer
5) A bottle containing N/10HCl

PROCEDURE

Take N/10 HCL up to the lowest mark in the diluting tube. Keep the diluting tube in the space provided in the box. Sterilize the fingertip using rectified spirit. Make a quick deep prick to get a moderately large drop of blood. Suck blood into haemoglobin pipette up to the 20 mark without any air bubble. Wipe off any blood sticking to the tip and sides of the pipette using cotton. Transfer the blood immediately into the acid taken in the diluting tube. Rinse the pipette two or three times with the acid and transfer in into the diluting tube. Mix and keep it undisturbed for 10 minutes for conversion of hemoglobin to acid haematin.

After 10 minutes, dilute the contents by adding distilled water, drop by drop and mixing the contents after each drop, with the stirrer, till the color matches with the color of the standard. Then take the reading at the lower meniscus both in gram per deciliter and percentage.

PRECAUTIONS

(1) There should not be any air bubble or clot in the blood column in the pipette
(2) Graduations on the diluting tube should not interfere with the colour matching
(3) The glass rod should be removed before colour matching and reading
(4) Take the reading at eye level without parallax error

DISADVANTAGES

(1) Individual variations in color matching
(2) Color of the standard fades with time
(3) Incomplete conversion of hemoglobin to acid haematin (sulf haemoglobin, metr haemoglobin and carboxy haemoglobin will not form acid haematin)

(4) Presence of proteins, lipids and bilirubin in plasma interfere with colour matching.

(5) Acid hematin is not in true stable solution; but is in suspension. So color of the solution will change with time. Due to lack of stable standard solution, acid hematin cannot be measured by photoelectric colorimetry.

Other methods

I. Other colorimetric methods:

(a) Direct matching: Inaccurate. Useful for rapid estimation of Hb of large no. of people during field survey.

(i) Talquist paper scale method

(ii) Haldane's method

(b) Indirect matching:

Alkali haematin method:

This is a better method for estimation of haemoglobin. Even carboxy haemoglobin, meth haemoglobin,and sulph haemoglobin can be detected by this method. But HbF is alkali resistant. So not useful for neonates.

Alkali denaturation test is used to estimate HbF. First blood is treated with alkali to denature all type of Hb except HbF and they are separated. Then the remaining HbF is estimated.

II. Gasometric method

This is based on the amount of gas bound by haemoglobin and then released.

1) Oxygen carrying capacity method

2) CO saturation method

III. Photometric method

Visual comparison or colour matching with the naked eye is subject to individual variations, which can be avoided by photo electric colorimetry.

The most accurate and preferred method of determining haemoglobin concentration is the cyanmet haemoglobin method using Drabkin's solution. This is the most widely used standard method. Drabkin's solution contains Dihydrogen potassium phosphate, potassium ferricyanide and potassium cyanide. Oxyhemoglobin, reduced hemoglobin, carboxyhemogloin are converted to cyanmethhemoglobin. Amount of light absorbed (peak at 450nm) is compared to that of a standard solution.

IV. By determining the iron content of blood: Hb concentration is calculated using the fact that 1gm Hb contains 3.35mg iron.

V. Physical methods

1) Making use of specific gravity. Specific gravity of blood is compared to that of copper sulphate solution of know specific gravity. From specific gravity, Hb concentration is assumed.

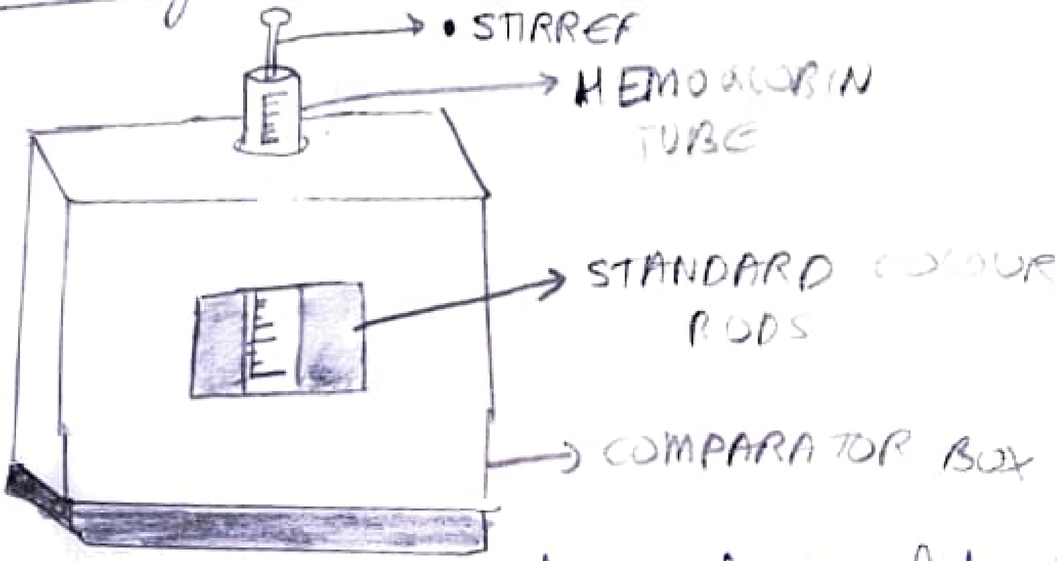
2) Spectroscopic method.

Normal values

Males	14-18gm/100ml
Females	12-16gm/100ml
New born	18-23gm/100ml

ANSWERS

1 Sahli's hemoglobinometer



2. The common anti-coagulant used in laboratory for hemoglobin estimation is EDTA

3 Anemia is a condition where the hemoglobin content of the blood is below the normal level of age and sex of person.

Thus an adult male is said to be anemic when his Hb falls below 13g/dl and an adult female is said to be anemic when the Hb falls below 11.5g/dl

C Classification of Anemia

(a) Depending upon the content

(i) Mild anemia – where Hb content is 10-11g%

(ii) Moderate anemia – where Hb content is 7-8g%

(iii) Severe anemia – where Hb content is 6g%

(b) Etiological classification of anemia

(i) Hemolytic anemia – due to increased destruction of RBC.

(ii) Anemia due to defective formation of RBC.

(iii) Post haemorrhagic anemia

Physiological variations : More in males, at high altitude (due to hypoxia), after exercise, in newborns. Less in females, pregnancy (due to hemodilution).

Hypoxia stimulates Erythropoietin secretion which stimulates erythropoiesis which in turn increases Hb content.

Pathological variations

Hb content is increased in polycythemia and hemoconcentration. Decreased in all types of anemia.

Functions of haemoglobin

1. Transport of oxygen
2. Transport of CO2
3. It acts as a buffer
4. Transport and inactivation of NO.
5. Gives colour to blood.

Result: Hemoglobin level of my own blood sample is ...16.............g/dL

Questions-

1. Draw and label Sahli's hemoglobinometer.
2. What is the common anticoagulant used in the laboratory for hemoglobin estimation?
3. Define Anemia. List out the classification of Anemia?

(c) classification of anemia depending on morphology:-

(i) Normocytic monochromatic anemia - acute haemorrhage aplastic anemia.

(ii) Macrocyte normochromic anemia - in pernicious anemic.

(iii) Microcytic hypochromic anemia - in iron deficiency anemia, thalassemia, chronic hemorrhage.

(iv) Microcytic normochromic anemia - in copper deficiency.

(v) macrocytic hypochromic anemia - in ~~pernicious~~ combined ~~anemic.~~ iron and folic acid deficiency

Experiment 6

Date : 16-01-23

OSMOTIC FRAGILITY

AIM

To determine the osmotic fragility of blood

PRINCIPLE

Osmotic fragility of red blood cells is assessed by adding blood to hypotonic saline solutions of decreasing concentrations.

APPARATUS

A series of 10 small test tubes (Kahn tubes), sodium chloride solution of varying strength from 0.25% - 0.7% at a difference of 0.5%, sterile lancet, wooden rack, cotton and spirit.

PROCEDURE

Place the 10 test tubes on the wooden rack labelled from 0.7% to 0.25%. Fill the tubes up to three fourth with sodium chloride solutions of decreasing strengths from 0.7% to 0.25% serially. Under sterile precautions, prick the finger tip and add one drop of blood to each test tube. Close the mouth of the test tube with the index finger and mix the contents gently by inverting the tube twice or thrice. Allow it to stand for one hour. Examine the tubes from higher to lower concentration.

OBSERVATION

In tubes with solution of higher concentration, blood settle down with clear colorless supernatants. As the concentration decreases gradually, supernatant start getting a reddish tinge. The strength of the sodium chloride in which the reddish tinge is first seen indicates the beginning of hemolysis (most fragile RBCs are lysed). The solution in which hemolysis is complete (all RBCs are lysed) is uniformly red and transparent without any sediment. In all the solutions of lower concentration the same picture is seen. Osmotic fragility is expressed as the range of saline concentration.

DISCUSSION

Osmotic fragility is the ease with which RBCs undergo lysis, when kept in hypotonic solution. Normal osmotic fragility is 0.45% - 0.35%.

When the red cell is placed in hypotonic saline, water is drawn in by the higher intracellular osmotic pressure of the red cells. Consequently, the red cells become spherical and ultimately the red cell membrane ruptures and hemoglobin (Hb) is released. This process of liberation of Hb from the red cells is known as hemolysis or laking of blood.

Variations

Conditions in which osmotic fragility in increased:

Physiological:

1. New born
2. Venous blood
3. Stored blood
4. Increase in temperature

Pathological:

1. Hereditary spherocytosis
2. Acquired spherocytosis
3. Snake envenomation eg:- Viper, Krait

Answers

1 Osmotic fragility test can be used to hel diagnose two heridatory conditions. Thalanem and Heredatory spherocytosis. It is done to check if RBCs will undergo hemolysis whe placed in different concentrations of saline solutions

② Anti-malaria drugs, snake venom, Penicillin, intravenous water infusion, Arsenic, Dapsone, Metal etc.

③ The walls of vein are thinner as compared to arterial wall and veins have more capacity to acquire blood. During CO_2 transport by venous blood, Cl^- ions move with H_2O (Chloride & Water shift) from plasma to RBC. That is why the volume of RBC in venous blood is more than RBC in arterial blood. Due to Cl^- shift, venous blood is having increased fragility than arterial blood.

④ Extended storage time caused increase in osmotic fragility. Maximum shelf life is upto 42 days. The hematocrit increased by 6.60% from day 0 to 42. Storage lesions and morphological changes in RBC membrane appeared to affect RBC during storage period

Conditions where osmotic fragility is decreased

1. Sickle cell anemia
2. Iron deficiency anemia
3. Thalassemia

Result : Hemolysis started at 0.45% NaCl
Hemolysis complete at 0.35% NaCl
Normal osmotic fragility is 0.45% - 0.35%

Questions

1. What is the role of the above test in clinical medicine?
2. Name a few hemolytic agents.
3. Venous blood is having increased fragility than arterial blood. Explain this.
4. Osmotic fragility increases in blood in blood bank over time. Why?